

Structure and Properties of Lignin From Five Distinct Biomasses

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Research

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Abstract

We have investigated the structural, thermal and morphological properties of lignin extracted from five distinct biomasses in order to establish the influence of the age of the source, environmental conditions during growth and quantity present. In fact, lignin is an integral part of cellulosic biomass and affects the structure, properties, processing and applications. Presence of lignin is critical for structural integrity but detrimental for converting biomass into new products. Aerial roots from banyan tree, sugarcane bagasse, bamboo, cornhusk and rice straw were selected to extract Klason lignin using the sulfuric acid approach. Extent of lignin in the samples varied from 9.14 to 23.77 %. Overall, compositional analysis showed that the lignin from the five sources had considerable variation, particularly in terms of the trace elements. Nevertheless, structure and properties of lignin extracted from five distinct biomasses was found to be independent of the age of the source, conditions during growth or quantity present in the biomass.

Introduction

Lignin is an integral part of cellulosic biomass and is one of the most sophisticated biomolecules. It is responsible for the strengthening of the plant cell wall, providing protection against microorganism and improving resistance to water and moisture (Lupoi et al. 2015). Lignin has a complex structure and is recalcitrant to most chemicals. It is broadly classified as woody type and herbaceous crop lignin (Zeng et al. 2013). According to the monomeric composition, lignin is sorted into phenolic p-coumaryl (H), coniferyl (G) and sinapyl alcohol (S) units. The type of lignin found in a particular plant determines its structure and properties. For instance, gymnosperms have G lignin whereas monocotyledons have all three types of lignin (Sette et al. 2011). It is not just the amount but the structure and functions of lignin that also change from source to source. Grabber et al. have reported that disturbances in the biosynthesis of lignin will result in generation of unusual precursors, which decrease degradability and increase hydrophobicity (Grabber 2005).

Although lignin is necessary for maintaining the plant structure, delignification is done for most applications. Removal of lignin is necessary to transform biomass into paper, textiles, ethanol and to most other products (Guna et al. 2019a; Guna et al. 2019b; Ilangovan et al. 2020; Ilangovan et al. 2018; Reddy and Yang 2005a; Reddy and Yang 2005b). However, controlled delignification is required to obtain high quality fibers for textile applications whereas near complete delignification is done to convert biomass into pulp or for production of ethanol (D.L. et al. 2017; Ilangovan et al. 2018; Reddy et al. 2007; Reddy and Yang 2009a; Reddy and Yang 2009b). Considerable efforts have been made to modify lignin and achieve better delignification and hence pulping efficiency (Prinsen et al. 2013). The amount of delignification is measured in terms of Kappa numbers and used to achieve the desired level of pulping. Chemical processes such as soda-AQ and soda-O₂ are done for delignification. Since plant materials contain up to 30% lignin and delignification is crucial, there is considerable amount of lignin available as residue or coproduct. Certainly, the properties of lignin also change depending on the kind of extraction.

Several studies have focused on studying the properties of lignin from different sources. In fact, structure, properties and process ability differ widely between sources and the diverse type of processing, and several researchers have attempted to understand the relationship between structures of lignin from various sources (Soukup et al. 2004; Vanholme et al. 2010). Lignins were extracted from wheat straw, sisal, abaca, hemp and jute and compared with organosolv lignin obtained from maple, birch or poplar and also with kraft lignin obtained from softwood (Boeriu et al. 2004). Lignins from all the sources studied were reported to have anti oxidative properties. FTIR was used to understand the structure dependent properties of softwood, mixed hardwood and several fibrous materials (Boeriu et al. 2004). In another study, milled wood lignin and organosolv lignin were fractionated sequentially to obtain lignin with different weight fractions (Park et al. 2018). Lignin with lower molecular weight had lower thermal stability but number of phenolic and methoxyl groups increased (Park et al. 2018). In addition to the source, the method of extraction of lignin was also different. Based on statistical analysis, it was concluded that FTIR could be used to readily determine the amount and type of lignin in various samples. Interestingly, all lignin samples showed radical scavenging activity with sisal and abaca being most effective. The level of antioxidant activity could also be predicted using FTIR spectra (Boeriu et al. 2004). In another study, Raman spectroscopy was employed to determine the structural differences between *Arabidopsis*, *Miscanthus* and poplar. It was suggested that structurally distinct lignin could be identified using Raman spectroscopy and that the signatures β -O-4 linkages were critical for the identification of different lignins (Perera et al. 2012). A formic acid and peroxy formic acid treatment were used to extract lignin from wheat straw, pine straw, alfalfa, kenaf and flax fiber (Watkins et al. 2015). Lignin obtained from alfalfa and flax provided higher enthalpy whereas wheat straw lignin has better thermal stability. The higher thermal resistance of lignin was considered to be suitable for replacement of phenolic resins (Watkins et al. 2015).

In addition, the type of pre-treatment combined with the extraction procedure used also affects the structure and properties of lignin. For instance, lignin obtained from oil palm fronds by ethanol organosolv process, using different pretreatment conditions, showed differences in phenolic hydroxyl content and their antioxidant activity (Hussin et al. 2014). Similarly, lignin has also been found to be affected by UV and other environmental parameters (Reddy et al. 2007). Considerable degradation of lignin was observed due to exposure to UV radiation. However, studies have also indicated that proportion of the different types of lignin (acetalated syringyl lignin and β -O-4 thioacidolysis yield) were responsible for the level of delignification. By controlling these parameters, it was suggested that better delignification and hence higher cellulosic yield could be achieved (Zhang et al. 2011).

Overall, previous studies have shown that structure and properties of lignin are affected by several parameters. In this study, we have chosen five distinct lignocellulosic sources and characterized the structure and properties of their lignin. The sources have been selected to be representative of different classes of plants with considerably dissimilar life span and location. Actually, banyan roots and bamboo have typical life span of more than 100 years compared to less than 6 months for rice straw and cornhusks. Similarly, rice and sugarcane are grown in considerably different environmental conditions than cornhusk or bamboo. Our intention is to understand if the type of plant, growing conditions and life

span affect the properties of lignin. Klason lignin was extracted from the five sources and fully characterized for its thermal behavior, composition, morphology and structural features. A detailed characterization is essential for the selection of future and suitable applications.

Materials And Methods

Materials

Aerial roots from Banyan tree, cornhusks, rice straw, bamboo and sugarcane bagasse were collected locally. Chemicals required for the study were purchased from local suppliers as analytical grade.

Lignin extraction

Raw materials were first made into powder in a grinder. About 5 grams of the powdered raw material was added into 25 mL of 72% (v/v) sulfuric acid. This mixture was allowed to stay for 2 hours at room temperature. Later, about 2700 mL of water was poured into the dissolved lignin and the solution was boiled under reflux for about 5 hours. Later the liquid was filtered, and the remaining solid fraction was collected as residual lignin. The lignin obtained was thoroughly washed multiple times using warm water to remove the acid. Weight percent of the extracted lignin was calculated based on the initial weight of the sample used for extraction and the final weight of lignin obtained.

Characterization

Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted on a Nicolet NEXUS 670 (Thermo-Nicolet, Waltham, MA) FTIR spectrometer using KBr powder. Measurements were performed from 400 to 4000 cm^{-1} , with a resolution of 4 cm^{-1} , and 64 scans were collected. The FTIR spectra obtained were analyzed using OMNIC software (Thermo Electron Corp).

X-ray photon spectroscopy (XPS) of the five different lignin samples were analyzed in a Thermo Fisher (model: ESCALAB 250 xi) spectrophotometer using AlK α radiation (1486.6 eV).

Inductively coupled plasma-mass spectrometry (ICP-MS) was performed by using a NEXION 300X (Perkin Elmer). About 100 mg of sample were mineralized in a Microwave Reaction System (Multiwave 3000, Anton Paar; mineralization program: 500W, 4 min; 1000W, 5 min; 1400W, 5 min) with 4.0 mL of nitric acid (VWR, Milan) and 1.0 mL of hydrogen peroxide (Fluka, Milan). Ten ppb of internal standards (Y, Re, Ge; Fluka, Milan) were added for the quantitative analysis.

Thermogravimetric analysis (TGA) was used to compare the thermal degradation behavior. TGA was performed on the lignin samples in a Perkin-Elmer STA 6000 (Norwalk, CT) calibrated with nickel, using a platinum pan. Samples (5-10 mg) were placed under nitrogen atmosphere and heated from 50 to 900 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C min}^{-1}$.

Crystalline structure was investigated by X-ray diffraction (XRD) on a D/max-2550 PC operating at the Cu K α wavelength of 0.15406 nm. A scanning rate of 0.06 deg/s was applied to record the diffraction pattern in the 2 θ range of 5–50°.

Morphology was studied by Field-Emission-Scanning Electron Microscopy (FE-SEM) and Transmission electron microscope (TEM). FESEM measurements were performed on a Hitachi S4800 high-resolution field emission scanning electron microscope with an accelerating voltage of 1.0 kV. Before measurements, the powder was coated with a thin layer of gold-palladium. Energy Dispersive X-ray Analysis (EDX) was carried out in the FESEM using the coupled EDX attachment. TEM measurements were carried out on a Zeiss Libra 120 transmission electron microscope. The accelerating voltage was set at 120 kV. The samples were prepared by placing a drop of the diluted capsules dispersion on a formvar-carbon-coated copper grid with 200 meshes.

Results And Discussion

Yield and composition

Table 1 summarizes the yields and compositional data for the five lignin samples. Amount of lignin extracted from the five sources was considerably different. While banyan provided the highest lignin content of about 23.77 %, cornhusk had the lowest lignin content. Bagasse, cornhusks and banyan roots had lignin content of 18.52, 9.14 and 23.77%, respectively. Based on the EDAX analysis, the chemical composition of lignin was found to be predominantly carbon (50-55%) and O (45-50%), except for rice straw. In rice straw, there was about 15% of silica and, consequently, only about 25% carbon (Nylese et al. 2015).

However, further study using ICP-MS study showed presence of several metals and the most abundant are reported in **Table 1**. The complete ICP-MS data, including metals present in trace amount, have been reported in the supporting information (**Table 1S**). Calcium was the most prominent among the different metals. It was highest in bagasse and cornhusk and lowest in rice straw. Magnesium ranged from 0.7 to 2.2 mg/g and, again, it was highest in bagasse and lowest in rice straw. Sodium and barium were present more consistently and varied from 0.2 to 0.5 mg/g and 0.01 to 0.02 mg/g, respectively. Among the heavy metals, iron is the most abundant with the highest amount in rice straw (**Table 1**). Content of Mn was significantly higher in lignin from straw rice than in the other samples. Unlike other metals, chromium was lowest in sugarcane but highest in cornhusk (**Table 1S**). The amount of trace elements should mainly be dependent on the conditions during growth. Bamboo and banyan were grown naturally whereas bagasse, rice and corn were cultivated. Extent and use of pesticides and composition of the water should also contribute to the variations in the presence of trace elements.

Table 1: Yields and compositional data for the five lignin samples

Sources	Life Span	Lignin (%)	EDX		ICP-MS ^(a)				
			C %	O %	metals (mg/g)				
			Ca	Mg	Al	Na	K		
Bagasse (S1)	6-9 months	18.52	64.41	34.57	4.7	2.2	0.1	0.5	0.2
Bamboo (S2)	Unlimited	19.51	63.01	35.49	1.8	1.0	0.08	0.3	0.1
Banyan (S3)	Unlimited	23.77	59.92	38.48	2.9	1.4	0.1	0.3	0.18
Cornhusk (S4)	4-6 months	9.14	67.37	31.67	3.3	1.3	0.18	0.2	0.11
Rice straw (S5)	3-6 months	17.71	58.33	31.74	1.4	0.7	0.66	0.2	0.16

- The complete composition of metals in lignin has been reported in the supporting information.

XPS showed that the functional groups on the surface of the lignins were similar (**Figure1**). A single peak was observed at energy of 37.5 eV. Strongest peak was produced by rice straw and the least intense peak was by bagasse. Compositional analysis showed that the lignin from the five sources had considerable variation, particularly in terms of the trace elements.

Based on the FTIR analysis lignin from all the five sources had similar chemical composition (**Figure2**). The broad peak between 3410-3460 cm^{-1} is due to the hydroxyl groups present in the phenolic and aliphatic structures in lignin (Boeriu et al. 2004). Another set of peaks around the 2917 and 2847 cm^{-1} region are due to the CH stretching and related to fatty acids reasonably present in lignin. Considerably strong peaks are seen at the 1750-1720 cm^{-1} wavelength, which are from the carbonyl/carboxyl groups. Although the intensity of the peaks at 1515 and 1426 cm^{-1} varies significantly between the lignin samples, these peaks should be from the C-H vibration and aromatic rings.

Crystalline structure

Lignin has relatively poor crystalline structure and produces a single prominent diffraction peak at about 22°, similar to that seen in cellulose. Lignin from rice straw has a relatively weaker X-ray diffraction pattern compared to the other four samples. This slight shift in the peak of lignin from rice straw was probably due to the changes in the composition (**Figure3**). It has been suggested that the amount of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units affects the structure and properties of lignin (Vanholme et al. 2010). Although the X-ray structure of lignin suggests that it is considerably amorphous, the high durability against natural degradation and recalcitrance of lignin to various chemicals and treatments should be due to the complex chemical structure.

Thermal degradation behavior

Degradation curves of the lignin samples reveals loss of water at about 100 °C. Main degradation of lignin occurs between 300-400 °C. Broader and smaller peak for the rice straw lignin suggests that the

lignin structure could be less cross linked or may contain lower amounts of the C-C interlinked bonds (Hussin et al. 2014). However, no correlation was observed between the age of the plant and thermal degradation of lignin. Lignin from banyan roots had similar degradation rates compared to cornhusk which has considerably shorter life (**Figure4**). Thermal resistance of the lignins should be adequate for processing into various products.

Morphology

Lignin in bagasse showed a layered structure whereas bamboo lignin was porous as seen from the TEM images (**Figure 5**). Particulate matter was observed on the surface of all the samples. At 10k magnification, bagasse lignin showed a relatively smooth and non-particulate surface. However, higher magnifications showed a layered structure for bagasse and banyan whereas the other three samples had particles on their surfaces. The size of the particles also appears to be different in each type of lignin. Micro-sized particulate aggregates were also observed when lignin and cellulose were simultaneously extracted from jute fibers (Ahuja et al. 2018). Similar variations in morphology have been reported by other researchers. For example, the distribution of lignin in several plants such as beech and spruce wood has been found to have a lamellar pattern and follow the direction of the cellulose microfibrillar structure (Fromm et al. 2003). Such lamellar pattern was not observed in any of the samples. However, a network structure was revealed in the cornhusk lignin sample whereas rice straw lignin showed aggregates of particles and the bagasse sample did not show any particular shape. The effect of these morphological differences on the properties of lignin and the reason for the different arrangements will be investigated further.

Conclusions

Amount of lignin present in bamboo, rice straw, cornhusk and bagasse vary considerably. Elemental composition of the lignins also had a wide range depending on the source. However, Lignin extracted from five distinct biomasses did not show major differences in terms of physical structure or thermal decomposition. It was also found that the properties of lignin were not dependent on the age of the source or the physical features of the biomass. Morphologically, some of the lignins were lamellar whereas others showed presence of particles. Lignin is known to be critical for the structural stability and functions of biomass. Since no major differences were observed in the lignins from five vastly different biomasses, it appears that the arrangement and functioning of lignin may be different when in biomass compared to studying the properties after extraction. An in situ analysis without any treatment or modification may provide a better understanding of the variations in the structure and properties of lignin in different biomasses.

Declarations

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Authors' contributions

Narendra Reddy designed the experiments. Manikandan Ilangovan and Vijaykumar Guna carried out the experiments. Paola Rizzarelli and Emanuele Francesco Mirabella helped in the characterization of the samples. Narendra Reddy drafted the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

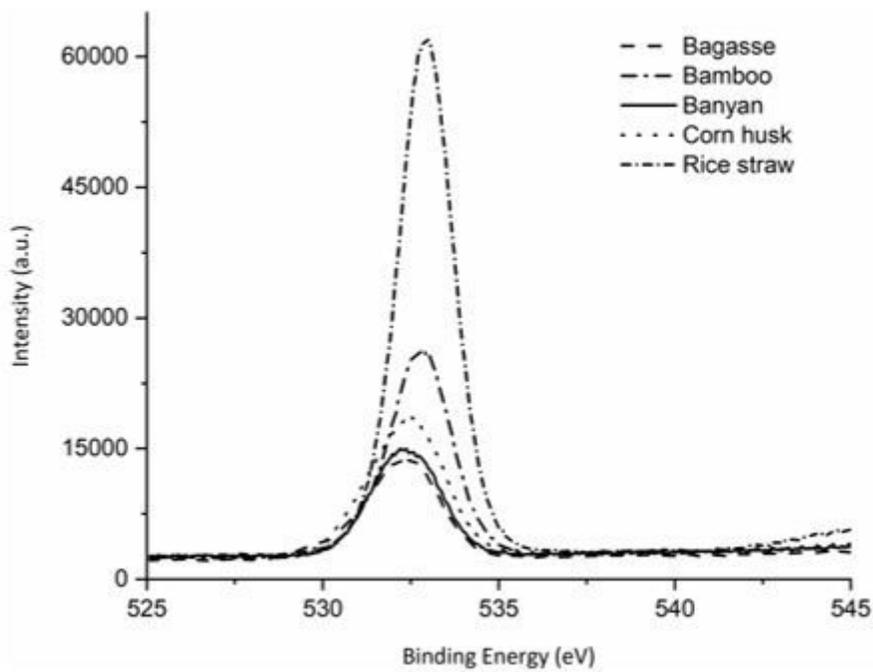


Figure 1

XPS spectra of the five lignin samples

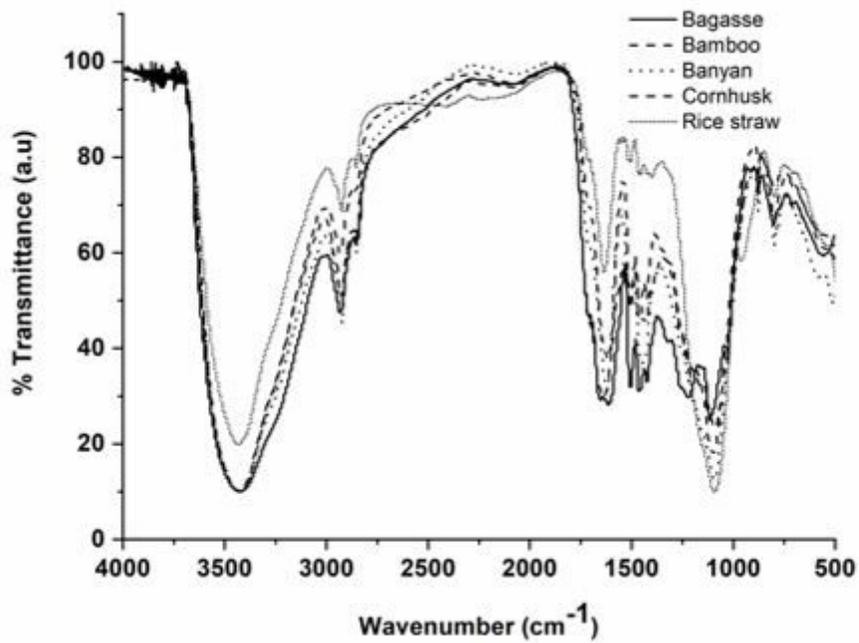


Figure 2

FTIR spectra of the five lignin samples

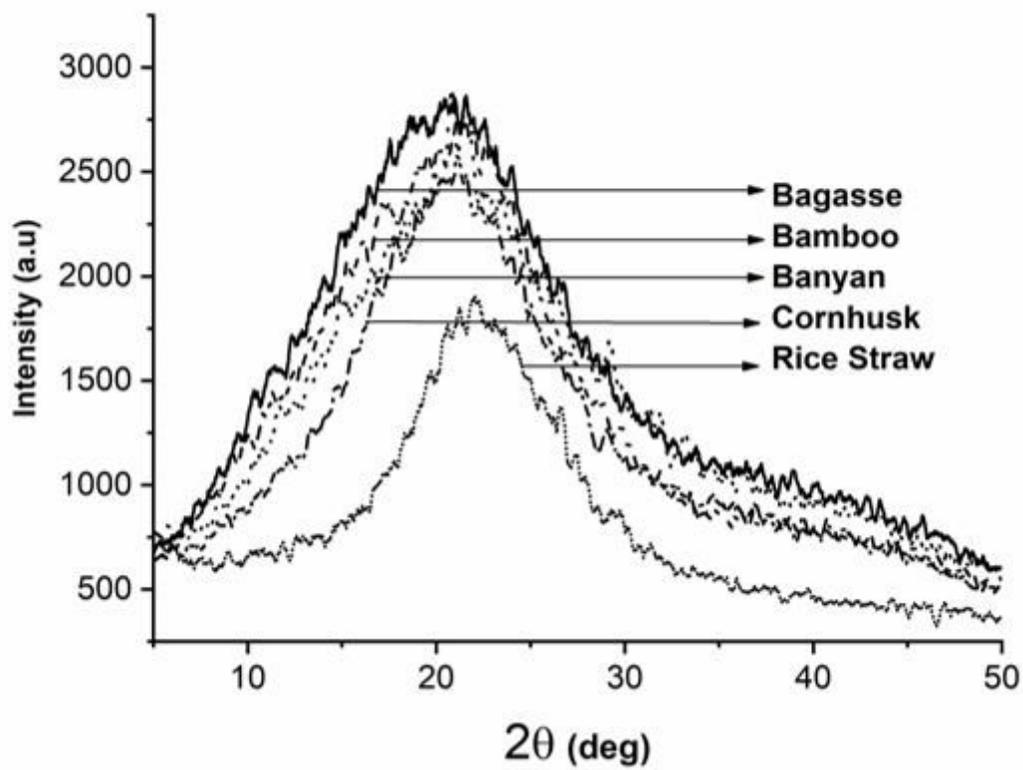


Figure 3

XRD patterns of the five lignin samples

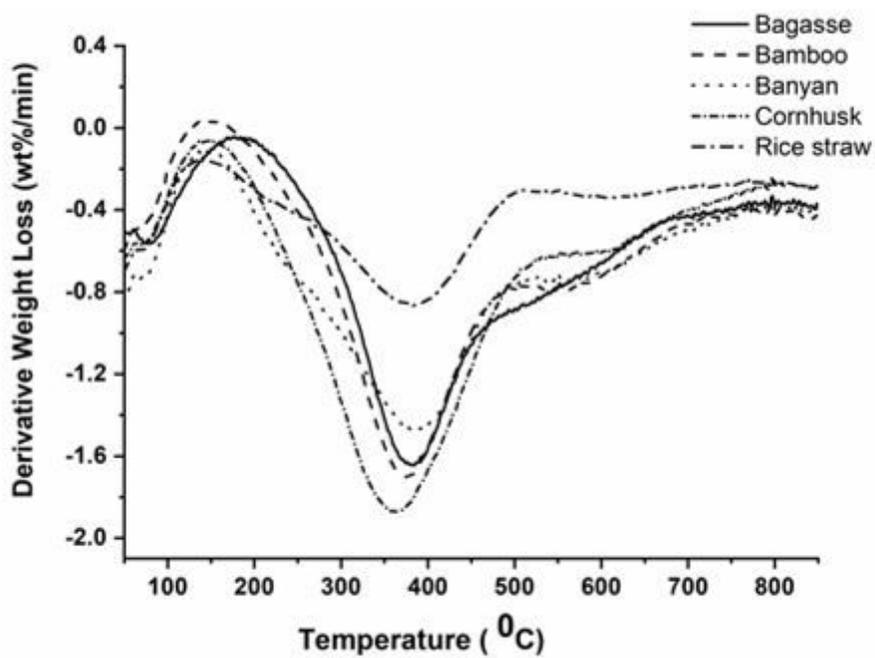


Figure 4

DTG profiles of the lignin from five samples

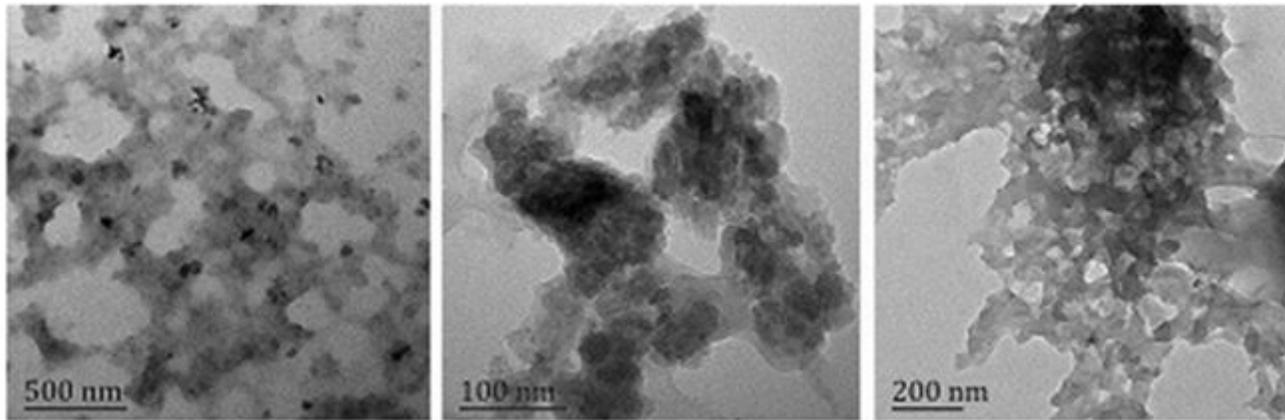


Figure 5

TEM images of the lignin in the biomass (Left-Cornhusk; Center-Rice straw and Right-Bagasse) show considerably different morphological arrangement

Supplementary Files

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